

Redescription and a significant range extension of *Rhacophorus napoensis* Li, Liu, Yu & Sun, 2022 (Amphibia, Anura, Rhacophorinae) with the first record from Vietnam

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https://zoobank.org/BF6A5C69-4EDC-444A-8D53-9A95FF0961D9

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Academic editor: Ben Wielstra ♦ Received 5 March 2024 ♦ Accepted 2 April 2024 ♦ Published 25 April 2024

Abstract

The Napo Treefrog *Rhacophorus napoensis* was described based on five male specimens from Napo County, Baise City, Guangxi Autonomous Region, China. Herein, we report on new findings and a range extension of this species based on an examination of preserved specimens collected from Bac Giang and Nghe An provinces, Vietnam. Futhermore, molecular analyses of specimens *Rhacophorus* spp. previously identified as *R. rhodopus* that were reported in Bac Giang, Nghe An as well as Thanh Hoa, and Ha Tinh provinces based on mitochondrial DNA supported the morphological findings. The Vietnam specimens have a pairwise divergence of less than 2% from those of the type series of *Rhacophorus napoensis* (based on the 16s rRNA mtDNA gene). Based on the new information, we confirm the presence of *R. napoensis* in Vietnam and update the diagnostic characters of this species and distribution. We suggest the species should be considered as of Least Concern (LC) following the IUCN's Red List categories. Further studies reassessing the populations of the *Rhacophorus bipunctatus* complex are required.

Key Words

morphology, mtDNA, new record, phylogeny, redescription, Rhacophorus bipunctatus complex, Vietnam



Introduction

Currently, the Treefrog of the genus *Rhacophorus* Kuhl & Van Hasselt, 1822 contains 44 nominal species recognized, distributed widely across South and Southeast Asia, including India, Bangladesh, Myanmar, Indochina, Malaysia, Indonesia, and Brunei, as well as extreme Southern and southwestern China (Jiang et al. 2019; Poyarkov et al. 2021; Frost 2024). Even so, the species diversity in the genus was indicated to be much underestimated and many cryptic species have not been described until now and this underscores the need for additional studies (Kropachev et al. 2022; Li et al. 2022). Presently, 16 nominal *Rhacophorus* species are recorded from Vietnam, including: R. annamensis Smith; R. calcaneus Smith; R. exechopygus Inger, Orlov & Darevsky; R. helenae Rowley, Tran, Hoang & Le; R. hoabinhensis Nguyen, Pham, Nguyen, Ninh & Ziegler; R. hoanglienensis Orlov, Lathrop, Murphy & Ho; R. kio Ohler & Delorme; R. larissae Ostroshabov, Orlov & Nguyen; R. marmoridorsum Orlov; R. orlovi Ziegler & Köhler; R. rhodopus Liu & Hu; R. robertingeri Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Nguyen & Geissler; R. trangdinhensis Kropachev, Evsyunin, Orlov & Nguyen; R. vanbanicus Kropachev, Orlov, Ninh & Nguyen; R. vampyrus Rowley, Le, Tran, Stuart & Hoang; and R. viridimaculatus Ostroshabov, Orlov & Nguyen (Poyarkov et al. 2021; Kropachev et al. 2022; Frost 2024).

Among the Treefrogs occurring in the Indochinese region of South-East Asia, the *Rhacophorus bipunctatus* complex is characterized by red web on feet, green or reddish-brown dorsal colour, presence of black spots at axillary region and skin folds above the anus, currenly including three species: R. bipunctatus Ahl, R. rhodopus, and R. napoensis Li, Liu, Yu & Sun (Bordoloi et al. 2007; Li et al. 2022). In the past few decades the phylogenetic relationship between R. bipunctatus and R. rhodopus has attracted considerable controversy (eg. Yu et al. 2008a; Li et al. 2012; Nguyen et al. 2014; Chan et al. 2018; Chen et al. 2022). The distribution of *R. bipunctatus* has now been restricted to Northeastern India, Bangladesh, Bhutan, and Northern and Western Myanmar, whereas R. rhodopus is known from Southern China, Central and Southern Myanmar, Thailand, Laos, Northern southwards Langbian Plateau Vietnam, Cambodia, and Peninsular Malaysia (Chan et al. 2018; Nguyen et al. 2020a; Poyarkov et al. 2021; this study).

The Napo Treefrog, *Rhacophorus napoensis* was recently described based on five males originating from Napo County, Baise City, Guangxi Autonomous Region, China (type locality) belonging to the *R. bipunctatus* complex (Li et al. 2022). To date, this species was known only from its type locality, which is approximately four kilometers northeast (straight line) of the Chinese/Vietnamese border. The species is characterized by: comparatively medium body size (SVL 38.6–43.6 mm in males); head width greater than head length, snout pointed, loreal region oblique, tympanum distinct, maxillary teeth

distinct, tongue cordiform, external single subgular vocal sac, tibiotarsal articulation reaches the snout, tibia length is greater than foot length and slightly greater than half of snout-vent length, and single outer metatarsal tubercle is flat (Li et al. 2022).

Futhermore, the Red-webbed Treefrog Rhacophorus rhodopus was reported to be widely distributed in the Northern southwards Langbian Plateau of Vietnam, including the following provinces: Ha Giang (Bac Me District [hereafter – Dist.]), Bac Giang (Tay Yen Tu Nature Reserve [hereafter – NR]), Ha Noi (Soc Son Dist.), Dien Bien [Muong Nhe NR], Lai Chau (Sin Ho Dist.), Lao Cai (Van Ban NR), Son La (Coipa NR, Sop Cop NR, Xuan Nha NR), Phu Tho (Xuan Son National Park [hereafter – NP]) Thanh Hoa (Xuan Lien NR), Nghe An (Pu Hoat NR, Pu Huong NR, Pu Mat NP), Ha Tinh (Vu Quang NP), Quang Binh (Phong Nha-Ke Bang NP), Quang Tri (Bac Huong Hoa NR), Thua Thien-Hue (Sao La NR), Quang Nam (Tay Giang Dist.), Kon Tum (Kon Plong Dist.), Gia Lai (Kon Ka Kinh NP, Kon Chu Rang NR), Lam Dong (Biduop-Nui Ba NP, Cat Tien NP, Di Linh Dist.), Binh Thuan (Nui Ong NR) (Nguyen et al. 2009; Hecht et al. 2013; Nguyen et al. 2014; Rowley et al. 2014; Nguyen et al. 2015b; Pham et al. 2016; Pham et al. 2017a,b; Luong et al. 2021; our data). However, the records of the Rhacophorus rhodopus in Vietnam are mainly based on morphological data, with very few molecular phylogeny investigations being examined. According to Nguyen et al. (2014) the Vietnamese populations from Bac Giang, Thanh Hoa, and Nghe An provinces are not conspecific with true *Rhacophorus rhodopus* from Yunnan Province, China (the province of the type locality of the species).

To address this, we examined specimens *Rhacophorus* spp. resembling *R. rhodopus* from Nghe An Province deposited in the Zoological Collections of Duy Tan University (DTU, Vietnam), Hong Duc University (HDU, Vietnam), and Zoological Museum of Lomonosov Moscow State University (ZMMU, Russia). Our results confirm that the specimens previously identified as *R. rhodopus* from Bac Giang and Nghe An provinces based both on morphological and molecular data, as well as the specimens from Thanh Hoa and Ha Tinh pronvices based on molecular data only, should be re-identified as *Rhacophorus napoensis*. We herein formally confirm the occurrence of *R. napoensis* in Vietnam, update the distribution of this species, provide additional data on its natural history and revise its diagnostic characters.

Materials and methods

Material examined

We examined 39 specimens that were previously registered as *Rhacophorus bipunctatus*, *R. napoensis*, and *R. rhodopus* from Northern and Central Vietnam, Northern and Central Myanmar, and Southern China (see Table 1, Fig. 1, and Suppl. material 1: table S1). Morphological

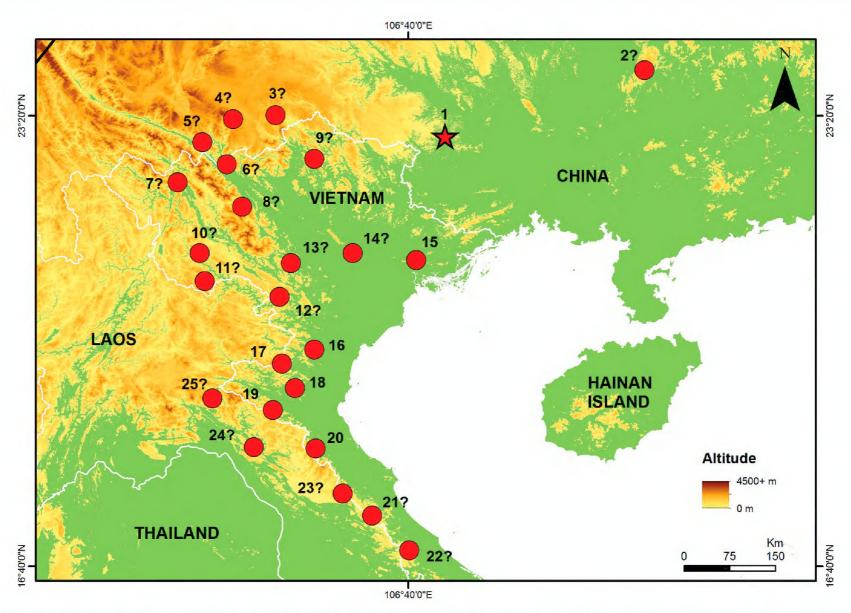


Figure 1. Distribution ranges of the species of the *Rhacophorus napoensis* in southern China and Vietnam. Notes: numbers indicate different localities where the species have been recorded (see Suppl. material 1: table S3 for the details of localities).

Table 1. Measurements (in mm) of the specimens of *Rhacophorus napoensis* from Vietnam.

| Specimen number | Sex | SVL | HW | HL | SL | ED | NS | EN | TYD | TYE | UEW | IOD | IN | FLL | HAL | FeL | TbL | FoL | TbW |
|-----------------------|------|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|------------|------|------|-------------|------|------|-----|
| HDU 1179 | M | 39.2 | 14.5 | 14.1 | 6.1 | 5.6 | 3.6 | 3.1 | 3.1 | 1.2 | 4.5 | 5.3 | 5.0 | 8.6 | 20.5 | 20.1 | 19.0 | 27.3 | 3.5 |
| HDU 1180 | M | 42.0 | 15.1 | 14.7 | 7.1 | 6.3 | 4.1 | 3.1 | 2.9 | 1.2 | 4.6 | 4.9 | 4.5 | 8.5 | 21.4 | 20.7 | 20.4 | 28.4 | 4.6 |
| DTU 84 | M | 39.5 | 14.2 | 14.1 | 6.1 | 5.9 | 3.5 | 2.7 | 3.0 | 1.0 | 4.5 | 4.8 | 4.7 | 8.3 | 19.1 | 19.0 | 18.6 | 25.4 | 2.9 |
| DTU 85 | M | 39.9 | 15.3 | 15.0 | 6.4 | 6.4 | 3.4 | 3.0 | 3.4 | 1.0 | 4.2 | 5.5 | 4.8 | 7.9 | 17.9 | 17.9 | 17.9 | 24.5 | 3.5 |
| DTU 87 | M | 38.6 | 14.2 | 14.1 | 5.7 | 5.4 | 3.3 | 2.5 | 4.2 | 1.3 | 4.1 | 4.8 | 4.2 | 8.0 | 19.4 | 18.6 | 18.5 | 27.1 | 3.8 |
| DTU 88 | M | 41.3 | 14.4 | 13.7 | 6.4 | 5.5 | 3.3 | 3.5 | 2.5 | 1.0 | 3.9 | 5.1 | 4.1 | 8.7 | 18.8 | 19.4 | 19.2 | 26.7 | 4.0 |
| DTU 89 | M | 43.2 | 15.9 | 15.8 | 6.6 | 5.9 | 3.4 | 3.3 | 3.1 | 1.2 | 4.2 | 4.9 | 4.7 | 9.6 | 20.4 | 19.9 | 19.6 | 27.0 | 3.9 |
| DTU 90 | M | 39.0 | 13.4 | 13.4 | 5.3 | 5.0 | 2.9 | 2.4 | 2.3 | 1.4 | 4.0 | 4.0 | 3.9 | 8.9 | 18.5 | 19.7 | 19.0 | 26.6 | 3.2 |
| ZMMU NAP-07414 | M | 40.9 | 14.8 | 14.6 | 6.3 | 5.9 | 3.5 | 3.1 | 3.2 | 1.2 | 4.4 | 5.0 | 4.6 | 8.7 | 19.7 | 19.6 | 19.2 | 26.8 | 3.7 |
| | Min | 38.6 | 13.4 | 13.4 | 5.3 | 5 | 2.9 | 2.4 | 2.3 | 1.0 | 3.9 | 4.0 | 3.9 | 7.9 | 17.9 | 17.9 | 17.9 | 24.5 | 2.9 |
| | Max | 43.2 | 15.9 | 15.8 | 7.1 | 6.4 | 4.1 | 3.5 | 4.2 | 1.4 | 4.6 | 5.5 | 5 | 9.6 | 21.4 | <i>20.7</i> | 20.4 | 28.4 | 4.6 |
| | Mean | 40.4 | 14.6 | 14.4 | 6.2 | 5.8 | 3.4 | 3.0 | 3.1 | 1.2 | 4.3 | 4.9 | 4.5 | 8.6 | 19.5 | 19.4 | 19.0 | 26.6 | 3.7 |
| | SD | 1.5 | 0.7 | 0.7 | 0.5 | 0.4 | 0.3 | 0.4 | 0.5 | 0.1 | 0.2 | 0.4 | 0.4 | 0.5 | 1.1 | 0.8 | 0.7 | 1.1 | 0.5 |
| HDU 1130 | F | 54.9 | 19.7 | 19.2 | 8.6 | 6.2 | 4.5 | 4.4 | 3.1 | 1.7 | 5.2 | 6.2 | 5.8 | 10.0 | 25.2 | 24.0 | 23.8 | 34.9 | 5.9 |
| DTU 86 | F | 55.8 | 20.0 | 19.0 | 7.9 | 6.4 | 4.3 | 4.0 | 3.5 | 1.9 | 5.2 | 6.1 | 5.6 | 11.4 | 25.4 | 25.2 | 25.1 | 35.7 | 4.8 |
| | Mean | 55.4 | 19.8 | 19.1 | 8.3 | 6.3 | 4.4 | 4.2 | 3.3 | 1.8 | 5.2 | 6.2 | <i>5.7</i> | 10.7 | 25.3 | 24.6 | 24.4 | 35.3 | 5.3 |
| | SD | 0.6 | 0.2 | 0.1 | 0.5 | 0.2 | 0.2 | 0.3 | 0.3 | 0.1 | 0.0 | 0.1 | 0.2 | 1.0 | 0.2 | 0.9 | 0.9 | 0.6 | 0.7 |

comparisons were based on literature data from: Wilkinson et al. (2005); Ohler and Delorme (2006); Bordoloi et al. (2007); Chan and Grismer (2010); Fei et al. (2009, 2012); Rowley et al. (2012); Hecht et al. (2013); Pham et al. (2016); Nguyen et al. (2020a); Li et al. (2022); and Fajri et al. (2023) (see Suppl. material 1: table S2).

Morphological analyses

Measurements were taken using digital calipers under a light dissecting microscope to the nearest 0.01 mm, subsequently rounded to 0.1 mm. The morphometrics of adults and character terminology follow Nguyen et al. (2020b) include SVL: snout-vent length, HL: head length (from back of mandible to tip of snout), HW: maximum head width (across angles of jaws), SL: snout length (from anterior corner of eye to tip of snout), NS: distance from nostril to the tip of snout, EN: distance from anterior corner of eye to nostril, IND: internarial distance, IOD: interorbital distance, ED: eye diameter, UEW: maximum width of upper eyelid, TD: tympanum diameter, TYE: distance from anterior margin of tympanum to posterior corner of eye, FLL: forearm length (from axilla to elbow), HAL: hand length (from elbow to tip of third finger), FL1–4: finger length I–IV, OPT: outer palmar

tubercle length, IPT: inner palmar tubercle length, NPL: nuptial pad length, FeL: femur length (from vent to knee), TbL: tibia length (from knee to tarsus), TbW: maximum tibia width, FoL: foot length (from tarsus to tip of fourth toe), TL 1–5: toe length I–V, IMT: inner metatarsal tubercle length, FD3: maximal diameter of disk of finger III, and TD4: maximal diameter of disk of toe IV. Terminology for describing eye colouration in living individuals followed Glaw and Vences (1997); subarticular tubercle formulas and webbing formula followed those of Savage (1975). All measurements were taken on the right side of the examined specimen. Sex was determined by gonadal inspection following dissection.

Molecular phylogeny

We focused on sequences for the mitochondrial 16S rRNA gene as it is phylogenetically informative for most tree frogs and has the largest availability of any gene for *Rhacophorus*. We synthesized previously published sequences of the Rhacophorus bipunctatus complex members from GenBank to estimate the phylogenetic relationships of the genus *Rhacophorus* and genetically identify samples referrable to R. napoensis. In addition, we sequenced a specimen (ZMMU NAP-07414) from Tay Yen Tu NR, Bac Giang Province, Vietnam. The new sequence has been deposited in GenBank under Accession No. PP391368. We used the sequences of *Zhangix*alus dennysi (Blanford) and Z. dugritei (David) to root the tree (GenBank accession numbers, voucher specimens, locality, and source information are summarized in Table 2).

Sequences were aligned using MAFFT 7.471 (Katoh and Standley 2013) with default parameters. Genetic divergences (uncorrected p-distance) were calculated in MEGA 11 (Tamura et al. 2021). The best substitution models were selected using the Akaike Information Criterion (AIC) in ModelFinder (Kalyaanamoorthy et al. 2017). Maximum likelihood (ML) phylogenetic analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015a) based on the TIM2+F+R4 model, and nodal support was estimated by 1,000 ultrafast bootstrap (UFB) replicates. Nodes with UFB values of 95 and above were considered significantly supported (Minh et al. 2013). Bayesian Inference (BI) was performed in MrBayes 3.2.7 (Ronquist et al. 2012) based on the GTR+F+I+G4 model. Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 1,000,000 generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in and then the remaining trees were used to estimate Bayesian posterior probabilities (BPPs). Nodes were considered well-supported if they had BPPs of 0.95 or higher (Huelsenbeck et al. 2001; Wilcox et al. 2002).

Other abbreviations. Dist.: District; Mt.: Mount; NP: National Park; NR: Natural Reserve.

Institutions and museums acronyms

CAS: California Academy of Sciences, San Francisco, USA; CIB: Herpetological Museum, Chengdu Institute of Biology, Chinese Academy of Sciences, Sichuan, China; **DTU**: Duy Tan University, Da Nang, Vietnam; GXNU: Guangxi Normal University, Guangxi, China; HDU: Hong Duc University, Thanh Hoa, Vietnam; **IEBR**: Institute of Ecology and Biological Resources, Hanoi, Vietnam; KIZ: Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences, Yunnan, China; NHMUK (formerly BMNH): The Natural History Museum, London, UK; NCSM: North Carolina Museum of Natural Sciences, North Carolina, USA; USNM: National Museum of Natural History, Smithsonian Institution, Washington D.C., USA; **ZMMU**: Zoological Museum of Lomonosov Moscow State University, Moscow, Russia.

Results

The ML and BI analyses recovered trees with similar topologies (Fig. 2). With respect to the position of *R. napoensis*, our phylogenetic results largely conform to those of Nguyen et al. (2014), Chan et al. (2018), Chen et al. (2022), Li et al. (2022). The sequences related to the *R. bipunctatus* complex formed six highly divergent lineages as follows: the lineage from Southern Yunnan, China represents the true *R. rhodopus*; the lineage from Northern Myanmar and Eastern India represents the true *R. bipunctatus*; the lineage from Guangxi, China and Northeastern Vietnam represents *R. napoensis*; and the other three lineages from Western Yunnan, China; Xizang, China; and Hainan, China as well as Gia Lai, Vietnam, respectively, represent three unnamed species (Fig. 2).

The sequences from Bac Giang, Thanh Hoa, Nghe An, and Ha Tinh provinces, Vietnam and the sequences of the type series of *R. napoensis* formed a highly supported clade, and there are two sub-clades within this clade. One sub-clade contains the sequences of the type series of *R. napoensis* and the sequences from Bac Giang Province; the other sub-clade contains the sequences from Thanh Hoa, Nghe An, and Ha Tinh provinces. The uncorrected pairwise distance between these two sub-clades was 1.8% (Table 3).

Furthermore, we also examined the morphology of *Rhacophorus* spp. specimens from Tay Yen Tu NR in Bac Giang Province as well as Pu Hoat NR Pu Huong NR, and Pu Mat NP in Nghe An Province and found morphological similarities with *R. napoensis*. These results support our hypothesis that previous records of *R. rhodopus* in Northern and North-Central Vietnam (Bac Giang, Thanh Hoa, Nghe An, and Ha Tinh provinces), should be referred to as *R. napoensis*. Therefore, we extend the distribution *Rhacophorus napoensis* to Vietnam and provide an expanded diagnosis and description.

Table 2. Sequences (16S) used in molecular analyses of this study. **Remark**: R = Rhacophorus, Z = Zhangixalus.

| Previously taxonomy | Proposed taxonnomy | | Locality | GenBank No. | Reference |
|-----------------------------|------------------------------|--------------------------------|--|----------------------|--------------------------------------|
| R. annamensis | R. annamensis | AMNH 161414 | Phong Nha-Ke Bang NP, Quang Binh, Vietnam | DQ283047 | Frost et al. (2006) |
| R. baluensis | R. baluensis | FM 235958 | Sabah, Borneo, Malaysia | KC961089 | Hertwig et al. (2013) |
| R. bipunctatus | R. bipunctatus | PUCZM/IX/SL 360 | Mizoram, India | MH087073 | Lalronunga & Ramliana unpublished |
| R. bipunctatus | R. bipunctatus | PUCZM/IX/SL 312 | Mizoram, India | MH087076 | Lalronunga & Ramliana unpublished |
| R. bipunctatus | R. bipunctatus | CAS 229913 | Putao, Kachin, Myanmar | JX219445 | Li et al. (2012) |
| R. bipunctatus | R. bipunctatus | CAS 235303 | Mindat, Chin, Myanmar | JX219444 | Li et al. (2012) |
| R. borneensis | R. borneensis | BORN 22411 | Maliau Basin, Sabah, Borneo, Malaysia | AB781694 | Matsui et al. (2013) |
| R. chuyangsinensis | R. calcaneus | KIZ 746 | Bidoup-Nui Ba NP, Lam Dong, Vietnam | JX219451 | Li et al. (2012) |
| R. catamitus | R. catamitus | ENS 14726 | Pesagi, Sumatra, Indonesia | KX398877 | O'Connell et al. (2018) |
| R. exechopygus | R. exechopygus | ZFMK 86409 | Phong Nha-Ke Bang NP, Quang Binh, Vietnam | GQ469980 | Unpublished |
| R. helenae | R. helenae | ZFMK 92544 | Tan Phu, Dong Nai, Vietnam | JQ288091 | Rowley et al. (2012) |
| R. hoabinhensis | R. hoabinhensis | VNMN A.2016.16 | Hang Kia-Pa Co NR, Hoa Binh, Vietnam | LC331097 | Nguyen et al. (2017) |
| R. indonesiensis | R. indonesiensis | MZB 23619 | Indonesia | AB983367 | Hamidy and Kurniati (2015) |
| R. kio | R. kio | VNMN 4110 | Kon Ka Kinh NP, Gia Lai, Vietnam | LC010589 | Nguyen et al. (2014) |
| R. laoshan | R. laoshan | 1705014 | Tianlin, Guangxi, China | MW149528 | Yuan et al. (2021) |
| R. lateralis | R. lateralis | SDB 2010.330 | Bygoor, Karnataka, India | KC571277 | Biju et al. (2013) |
| R. malabaricus | R. malabaricus | / FNG 16162 | Madikeri, Karnataka, India | AB530549 | Hasan et al. (2014) |
| R. margaritifer | R. margaritifer | ENS 16162 | Tilu, Java, Indonesia | KX398889 | O'Connell et al. (2018) |
| R. modestus | R. modestus | ENS 16853 | Utara, Sumatra, Indonesia | KX398904 | O'Connell et al. (2018) |
| R. napoensis | R. napoensis | GXNU YU000169 | Napo, Guangxi, China | ON217794 ON217795 | Li et al. (2022) |
| R. napoensis | R. napoensis | GXNU YU000170 GXNU YU000171 | Napo, Guangxi, China | ON217795 ON217796 | Li et al. (2022) |
| R. napoensis | R. napoensis | GXNU YU000171 | Napo, Guangxi, China Napo, Guangxi, China | ON217790 ON217797 | Li et al. (2022) |
| R. napoensis | R. napoensis R. napoensis | GXNU YU000172 | Napo, Guangxi, China | ON217797 ON217798 | Li et al. (2022) Li et al. (2022) |
| R. napoensis R. rhodopus | R. napoensis | VNMN 4118 | Tay Yen Tu NR, Bac Giang, Vietnam | LC010605 | Nguyen et al. (2014) |
| R. rhodopus | R. napoensis | VNMN 4118 VNMN 4119 | Pu Huong NR, Nghe An, Vietnam | LC010605 | Nguyen et al. (2014) |
| R. rhodopus | R. napoensis | VNMN 4119 VNMN 4122 | Xuan Lien NR, Thanh Hoa, Vietnam | LC010607 | Nguyen et al. (2014) |
| R. rhodopus | R. napoensis | VNMN 4121 | Xuan Lien NR, Thanh Hoa, Vietnam | LC010608 | Nguyen et al. (2014) |
| R. rhodopus | R. napoensis | VNMN 4120 | Pu Huong NR, Nghe An, Vietnam | LC010609 | Nguyen et al. (2014) |
| R. bipunctatus | R. napoensis | AMNH 161418 | Huong Son, Vu Quang NP, Ha Tinh, Vietnam | AY843750 | Faivovich et al. (2005) |
| R. napoensis | R. napoensis | ZMMU NAP-07414 | | PP391368 | This study |
| R. nigropalmatus | R. nigropalmatus | KUHE 53935 | Sarawak, Borneo, Malaysia | AB781701 | Matsui et al. (2013) |
| R. norhayatiae | R. norhayatiae | KUHE UNL | Endau Rompin, Johor, Malaysia | AB728191 | Kuraishi et al. (2013) |
| R. orlovi | R. orlovi | VNMN 3067 | Huong Son, Vu Quang NP, Ha Tinh, Vietnam | LC010598 | Nguyen et al. (2014) |
| R. pardalis | R. pardalis | FMNH 273243 | Sarawak, Bintulu, Malaysia | JX219454 | Li et al. (2012) |
| R. poecilonotus | R. poecilonotus | ENS 16480 | Utara, Sumatra, Indonesia | KX398920 | O'Connell et al. (2018) |
| R. pseudomalabaricus | R. pseudomalabaricus | | Kerala, Kadalar, India | KC593855 | Biju et al. (2013) |
| R. reinwardtii | R. reinwardtii | ENS 16447 (UTA) | Bandung, Sumatra, Indonesia | KY886335 | O'Connell et al. (2018) |
| R. rhodopus | R. rhodopus | SCUM 060692L | Mengyang, Yunnan, China | EU215531 | Li et al. (2008) |
| R. rhodopus | R. rhodopus | KUHE 47794 | Jinghong, Yunnan, China | LC386573 | Matsui et al. (2019) |
| R. rhodopus | R. rhodopus | Lc0805109 | Lvchun, Yunnan, China | JX219440 | Li et al. (2012) |
| R. rhodopus | R. rhodopus | KIZ 60821037 | Puer, Yunnan, China | EF564572 | Yu et al. (2008) |
| R. robertingeri | R. robertingeri | VNMN 3446 | Kon Plong, Kon Tum, Vietnam | LC010615 | Nguyen et al. (2014) |
| R. spelaeus | R. spelaeus | IEBR A.2011.1 | Khammouan, Lao | LC331095 | Nguyen et al. (2017) |
| R. translineatus | R. translineatus | Rao 6237 | Motuo, Xizang, China | JX219449 | Li et al. (2012) |
| R. tuberculatus | R. tuberculatus | KIZ 14154 | Motuo, Xizang, China | MW111522 | Wu et al. (2021) |
| R. vampyrus | R. vampyrus | VNMN 4125 | Hon Ba NR, Khanh Hoa, Vietnam | LC010616 | Nguyen et al. (2014) |
| R. rhodopus | Rhacophorus sp1 | KIZ 060821175 | Yongde, Yunnan, China | EF564573 | Yu et al. (2008) |
| R. rhodopus | Rhacophorus sp1 | KIZ 589 | Longling, Yunnan, China | EF564578 | Yu et al. (2008) |
| R. rhodopus | Rhacophorus sp1 | KIZ 060821248 | Jindong, Yunnan, China | EF564575 | Yu et al. (2008) |
| R. rhodopus | Rhacophorus sp1 | Loc 08007018 | Longchuan, Yunnan, China | JX219439 | Li et al. (2012) |
| R. rhodopus | Rhacophorus sp2 | L06245 | Motuo, Xizang, China | JX219441 | Li et al. (2012) |
| R. rhodopus | Rhacophorus sp2 | L062456 | Motuo, Xizang, China | JX219442 | Li et al. (2012) |
| R. rhodopus | Rhacophorus sp2 | / | Motuo, Xizang, China | OK165559 | Chen et al. (2022) |
| R. rhodopus | Rhacophorus sp2 | / | Motuo, Xizang, China | OK181853 | Chen et al. (2022) |
| R. rhodopus | Rhacophorus sp3 | SN030035 | Hainan, China | EF564579 | Yu et al. (2008) |
| R. rhodopus | Rhacophorus sp3 | SN030036 | Hainan, China | EF564580 | Yu et al. (2008) |
| R. rhodopus | Rhacophorus sp3 | SN 030035 | Hainan, China | EU215529 | Li et al. (2008) |
| R. rhodopus | Rhacophorus sp3 | / | Limu Mt., Hainan, China | EF646369 | Yu et al. 2007 |
| R. rhodopus | Rhacophorus sp3 | / | Limu Mt., Hainan, China | EF646370 | Yu et al. 2007 |
| R. bipunctatus | Rhacophorus sp3 | ROM 29944 | Tran Lap, Gia Lai, Vietnam | AF458144 | Wilkinson et al. (2002) |
| R. bipunctatus | Rhacophorus sp3 | FMNH 253114 | An Khe, Gia Lai, Vietnam | GQ204716 | Meegaskumbura et al. (2010) |
| R. rhodopus | Rhacophorus sp3 | VNMN 4117 | K'Bang, Gia Lai, Vietnam | LC010604 | Nguyen et al. (2014) |
| Outgroup | • | | | | |
| 0 1 | | | | | |
| Z. dennysi | Z. dennysi | SCUM 060401L | Shaoguan, Guangdong, China | EU215545 | Li et al. (2008) |

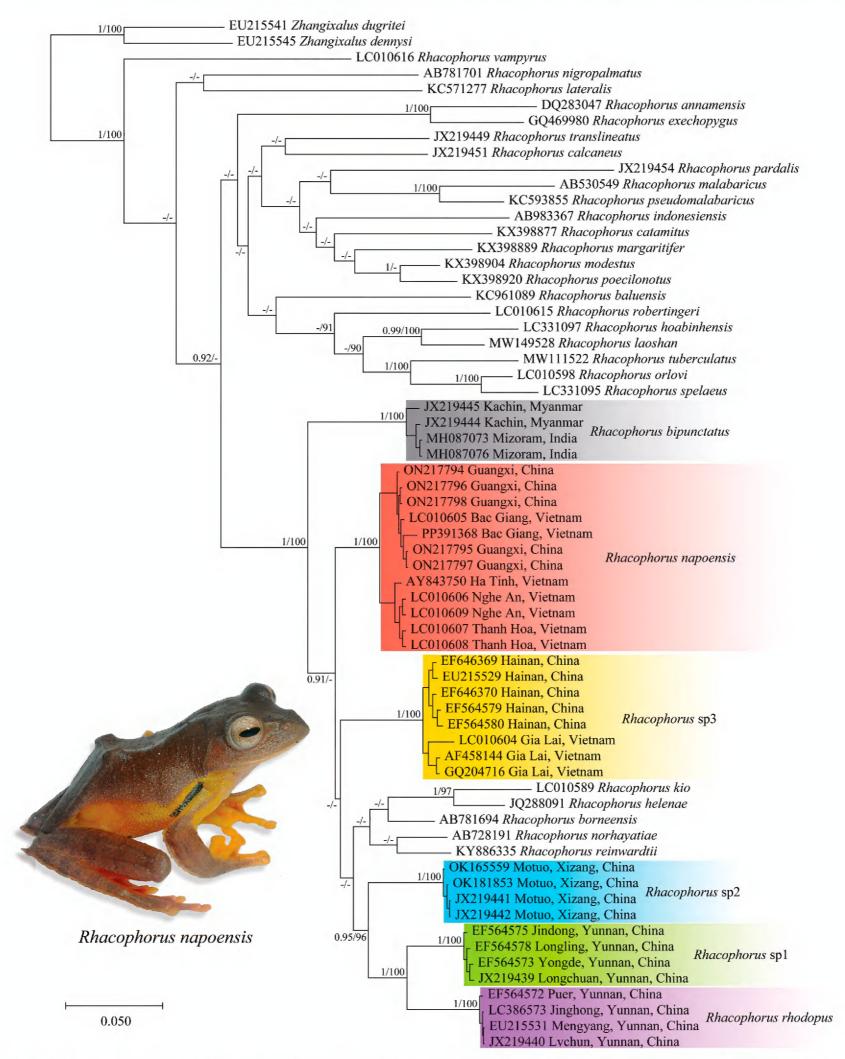


Figure 2. Maximum Likelihood (ML) tree of species in the genus *Rhacophorus* derived from an analysis of 16s mitochondrial DNA gene sequences. For voucher specimen information and GenBank accession numbers see Table 2. Numbers at tree nodes correspond to BI PP support values/ML UFBS, respectively. Photograph by N.A. Poyarkov.

Table 3. Uncorrected p-distances (%) of the 16S rRNA sequences within the *Rhacophorus bipunctatus* species complex.

| No | Species | 1 | 2 | 3 | 4 | 5 | 6 |
|----|---|-----|-----|-----|-----|-----|-----|
| 1 | Rhacophorus napoensis | | | | | | |
| | (Guangxi in China & Bac Giang in Vietnam) | | | | | | |
| 2 | Rhacophorus napoensis | 1.8 | | | | | |
| | (Ha Tinh, Nghe An, Thanh Hoa in Vietnam) | | | | | | |
| 3 | Rhacophorus bipunctatus | 7.3 | 6.5 | | | | |
| 4 | Rhacophorus rhodopus | 7.8 | 7.0 | 9.0 | | | |
| 5 | Rhacophorus sp1 | 7.1 | 7.0 | 7.6 | 5.0 | | |
| 6 | Rhacophorus sp2 | 6.4 | 4.9 | 8.8 | 7.2 | 6.0 | |
| 7 | Rhacophorus sp3 | 5.8 | 5.5 | 8.3 | 6.7 | 7.4 | 6.2 |

Taxonomic account

Rhacophorus napoensis Li, Liu, Yu & Sun, 2022

Table 1, Figs 3, 4

Holotype. GXNU YU000172, adult male collected by Shuo Liu on 25 March 2019.

Type locality. Napo County, Baise City, Guangxi Autonomous Region, China (23°1'20"N, 105°50'58"E; elevation 1032 m a.s.l.).

Chresonymy. Rhacophorus rhodopus (non Rhacophorus rhodopus Liu & Hu, 1969) – Nguyen et al. (2008: 364, in part); Nguyen et al. (2009: 181, in part); Hecht et al. (2013: 526–527, in part); Nguyen et al. (2014: 276–277, in part); Pham et al. (2016: 37–38, in part); Poyarkov et al. (2021: 61–62, in part).

Rhacophorus napoensis Li, Liu, Yu & Sun (2022: 130). Suggested name in Vietnamese. Éch cây Na pha

Specimens examined (n= 11). Two adult males HDU 1179–80 from Pu Hoat NR, Que Phong Dist., Nghe An Province, Vietnam (ca. 19.66313°N, 104.78185°E; elevation 1360 m a.s.l) collected by V.Q. Dau et al. on 12 April 2011. One adult female HDU 1130 from Khe Co area in Pu Huong NR, Chau Cuong Dist., Nghe An Province, Vietnam (19.33155°N, 105.0016°E, elevation 645 m a.s.l.) collected by V.Q. Dau on 22 July 2013. One adult male ZMMU NAP-07414 from Khe Cam I area within Tay Yen Tu NR, Son Dong Dist., Bac Giang Province, Vietnam (21.18058°N, 106.72683°E, elevation 181 m a.s.l.) collected by N.A. Poyarkov on 07 July 2017. Four adult males DTU 84–85, 87–88 and one adult female DTU 86 from Khe Moi area within Pu Mat NP, Con Cuong Dist., Nghe An Province, Vietnam (ca. 18.917446°N, 104.773233°E; elevation 900 m a.s.l) collected by T.V. Nguyen on 27 August 2018. Two adult males DTU 89-90 from Khe Bu area within Pu Mat NP, Tam Quang Dist., Nghe An Province, Vietnam (ca. 19.037120°N, 104.605944°E; elevation 760 m a.s.l) collected by T.V. Nguyen on 12 February 2019.

Revised diagnosis. Medium-sized within genus *Rhacophorus* (SVL 38.6–43.2 mm in males, 54.9–55.8 mm in females); snout pointed, projecting beyond margin of lower jaw in ventral view, and the tip has a distinct bulge; tympanum distinct, rounded; maxillary teeth distinct; tongue cordiform, notably notched posteriorly; external single subgular vocal sac; the tibiotarsal articulation reaches the snout; entire web between fingers and toes; single inner metatarsal tubercle, flat; banding exists in dorsal surface of limbs posterior part of dorsum; two to three black spots at axillary region; webbing not black; and dorsum reddish brown with or without small black spots (dorsum hoary in color with numerous black spots after preservation per original diagnosis); ventral yellowish white (data from Li et al. (2022) and this study).

Comparisons. We summarize the main characters separating *Rhacophorus napoensis* from the *R. bipunctatus* and *R. rhodopus* in Suppl. material 1: table S2. *Rhacophorus napoensis* differs from *R. bipunctatus* by having: lower maximum SVL in both sexes (43.2 mm in males, 55.8 mm in females vs. 50.4 mm in males, 59.1 mm in females), absent yellow pigmentation at the upper and lower portions of iris (vs. present, see Suppl. material 1: fig. S1), ventral coloration yellowish white (vs. bright yellow), dorsal coloration brown or reddish brown (vs. green or brown), snout pointed, and the tip has a distinct bulge (vs. broad and pointed), loreal region oblique (vs. concave), tympanum distinct (vs. slight indistinct), tongue cordiform, notably notched posteriorly (vs. round, slight notched posteriorly),

two to three black spots at axillary region (vs. one big and one small black spot at axillary region). *Rhacophorus napoensis* differs from *R. rohdopus* by having: larger body size in males (SVL 38.6–43.2 mm [avg. 41.41 mm] vs. 31.2–38.0 mm [avg. 35.16 mm]), slightly lower ratio TbL/SVL in both sexes (0.45–0.51 [avg. 0.48] in males, 0.43–0.46 [avg. 0.45] in females vs. 0.47–0.55 [avg. 0.51] in males, 0.44–0.50 [avg. 0.47], ventral coloration yellowish white (vs. bright yellow), tongue cordiform, notably notched posteriorly (vs. narrow and long, deeply notched posteriorly), external single subgular vocal sac (vs. internal single), the tibiotarsal articulation reaches the snout (vs. reaches the eye), two to three black spots at axillary region (vs. one black or dark round spot at axillary region).

Futhemore, *Rhacophorus napoensis* can be rapidly distinguished from most of the closely-related species of the R. reinwardtii complex (includes: R. borneensis Matsui, Shimada & Sudin, R. helenae Rowley, Tran, Hoang & Le, R. kio Ohler & Delorme, R. norhayatiae Chan & Grismer, and R. reinwardtii (Schlegel)) by having a distinctly smaller body size in both sexes (SVL 38.6–43.2 mm in males, 54.9–55.8 in females vs. 50.1 mm male, 62 mm in females in R. borneensis (data from Matsui et al. 2013); 72.3–85.5 mm [avg. 77.7 mm] in males, 89.4–90.7 mm [avg. 90.1 mm] in females of the R. helenae (data from Rowley et al. 2014); 66.6–69.5 mm [avg. 68.2 mm] in males, 78.0–88.9 mm [avg. 84.6 mm] in females of *R. kio* (data from Ohler & Delorme, 2006; Fajri et al. 2023); 60.6–64.7 mm [avg. 61.9 mm] in males, 75.7–83.0 mm [avg. 80.3 mm] in females of the R. norhayatiae (data from Chan and Grismer 2010); and 41.6–52.5 mm [avg. 46.4 mm] in males, 66.6–74.8 mm [avg. 70.7 mm] in females of the R. reinwardtii (data from Fajri et al. 2023); the absence of black spots/pigmentation on webs (vs. present in all five species), brown dorsum coloration in life (vs. green in all five species), and a significantly different color pattern on flanks.

Description based on examined specimens from **Vietnam (n=11).** SVL 38.6–43.6 mm in males (n=9), 54.9–55.8 mm in females (n=2); habitus relatively slender; head almost equal or slight width greater than long (HW/HL 1.00–1.05 in males, 1.04–1.05 in females); snout pointed in dorsal view and slightly acuminate in profile, protruding from the margin of the lower jaw; eyes relatively larger, slightly protuberant, pupil horizontal, eye diameter slightly equal to snout length (ED/SL 0.86–1.00 in males, 0.72–0.82 in females), pineal ocellus absent. Top of head flat, canthus rostralis rounded and distinct; loreal region oblique; nostril round, small, closer to eye than to eye tip of snout (NS/EN 0.94-1.32 in males, 1.02-1.07 in females); interorbital distance slightly greater than internarial distance (IOD/IN 1.02-1.24 in males, 1.07-1.10 in females) and upper eyelid width (IOD/UEW 1.00-1.31 in males, 1.18–1.19 in females). Tympanum and supratympanic fold distinct. Vomerine teeth present; tongue cordiform, attached anteriorly, notably notched posteriorly; choanae oval; external single subgular vocal sac, vocal sac openings at bottom of the mouth on both sides.

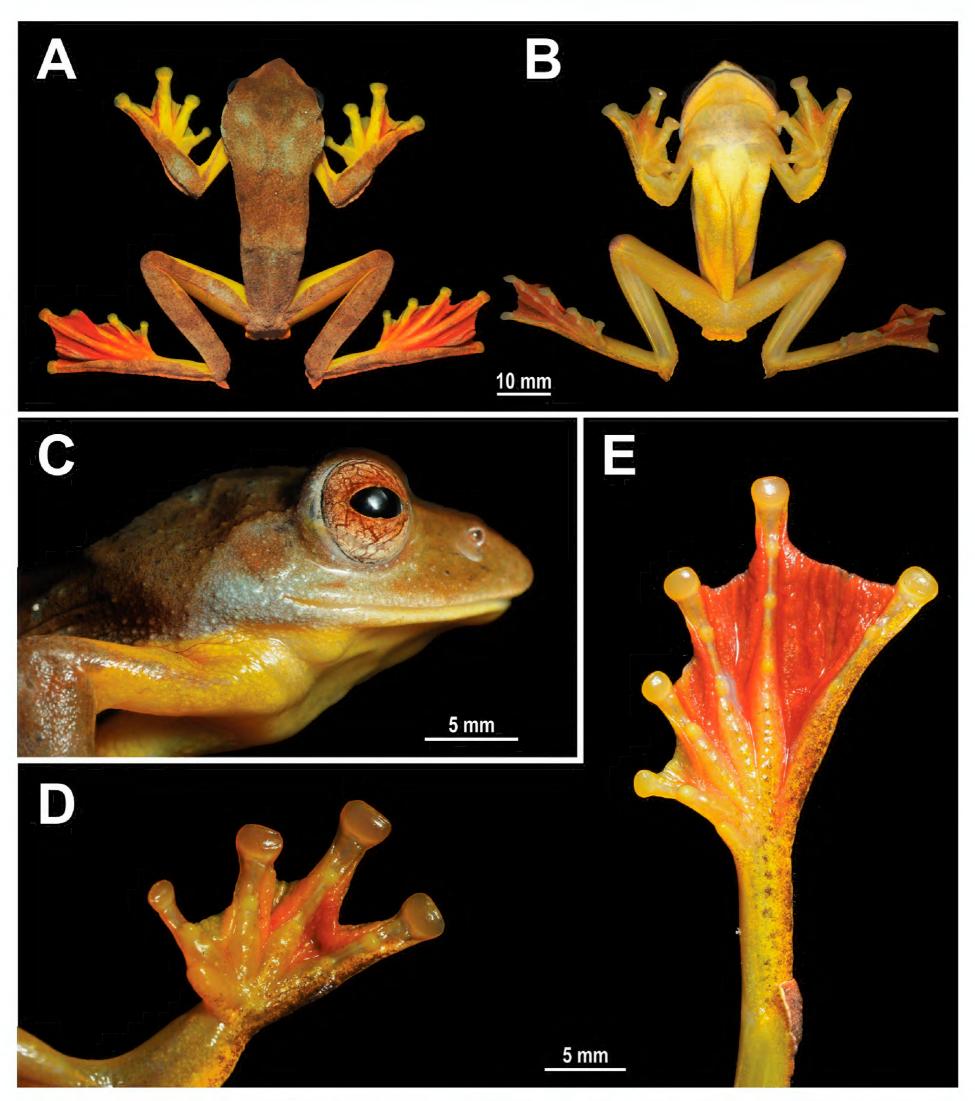


Figure 3. Morphological details of *Rhacophorus napoensis* from Tay Yen Tu NR, Bac Giang, Vietnam (ZMMU NAP-07414, adult male) **A**. Dorsal aspect; **B**. Ventral aspect; **C**. Head in lateral aspect; **D**. Volar view of left hand; **E**. Plantar view of left foot. Photographs by N. A. Poyarkov.

Forelimbs: Forearm length ca. two times shorter than hand length (FLL/HAL 0.40–0.48 in males, 0.40–0.45 in females). Fingers short, tips of all fingers expanded into discs; entire web between fingers; relative finger lengths: I<II<IV<III; subarticular tubercles on fingers distinct, rounded and prominent, formula: 1, 1, 2, 2; supernumerary tubercles below the base of finger absent; single thenar (inner metacarpal) tubercle large, oval, distinct; nuptial pad present on finger I in adult males.

Hindlimbs: thigh slightly longer than tibia (FeL/TbL 1.00–1.06 in males, 1.00–1.01 in females), approximately four to five times longer than wide (TbL/TbW 4.43–6.41 in males, 4.06–5.20 in females); tibiotarsal articulation of adpressed limb reaching snout; foot longer than tibia (TbL/FoL 0.68–0.73 in males, 0.68–0.70 in females). Relative toe lengths: I<II<III<V<IV; tarsal fold present; tips of all toes expanded into discs; entire webbing between toes; subarticular tubercles on toes oval and promi-

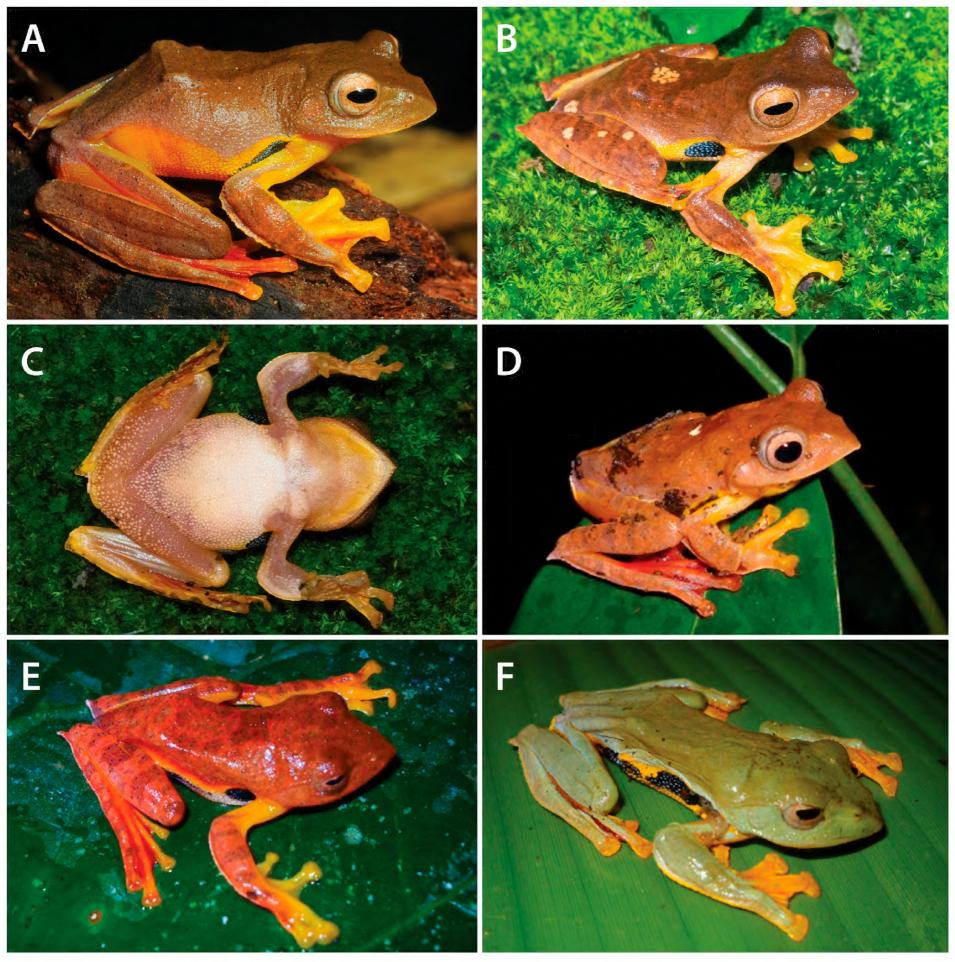


Figure 4. Alive specimens of *Rhacophorus napoensis*: from Tay Yen Tu NR, Bac Giang, Vietnam (**A**. ZMMU NAP-07414, adult male); from Pu Mat NP, Nghe An, Vietnam (**B**, **C**. DTU 89; adult male); from Pu Hoat NR, Nghe An, Vietnam (**D**, **E**. HDU 1179-1180, two adult males, respectively); from Pu Huong NR, Nghe An, Vietnam (**F**. HDU 1130, adult female). Photographs by: N. A. Poyarkov (**A**), T.V. Nguyen (**B**, **C**), and V.Q. Dau (**D**, **F**).

nent, formula: 1, 1, 2, 3, 2; supernumerary tubercle below the base of toe absent; inner metatarsal tubercle rounded, prominent, and small; outer metatarsal tubercle absent.

The skin of dorsal throat, ventral part of tibia, foot and tarsus smooth; the skin of chest, venter, vent and thigh rough and granular; some warts are found around the vent and flanks; dermal fringe along joint, vent and the outer sides of limbs. Cloacal dermal fringe present.

Colouration in life (Figs 3, 4): Dorsum reddish brown or gray brown with small black spots; two or three large black blotches on axilla and flanks present; transverse bands on hind limbs absent or indistinct; ventral yellowish

white; webbing of fingers and toes reddish orange without black spotted. Iris copper or pale yellow.

Distribution (Fig. 1). *Rhacophorus napoensis* was previously known only from Napo County, Baise City, Guangxi Autonomous Region, China (Li et al. 2022). We here report on the first records for Vietnam including provinces, namely: Bac Giang (Tay Yen Tu NR), Thanh Hoa (Xuan Lien NP), Nghe An (Pu Hoat NR, Pu Huong NR, and Pu Mat NP), and Ha Tinh (Vu Quang NP) previously reported as *Rhacophorus rhodopus*. The new location in Huong Son area within Vu Quang NP. is ca. 520 airline kilometers south of the type locality. Given its

geographic proximity, it likely occurs in several secondary and evergreen forests of Northern and North-Central Vietnam, Central Laos as well as Southern China; in particular, the records previously reported as *Rhacophorus rhodopus* from Ha Giang (Bac Me Dist.), Ha Noi (Soc Son Dist.), Lai Chau (Sin Ho Dist.), Lao Cai (Van Ban NR), Son La (Copia NR, Sop Cop NR, Xuan Nha NR); Phu Tho (Xuan Son NP); Quang Binh (Phong Nha-Ke Bang NP); Quang Tri (Bac Huong Hoa NR) provinces of Northern and North-Central Vietnam; Khammouan, Bolikhamxay, and Xiangkhoang provinces of Central Laos, as well as from southeastern Yunnan Province (Wenshan Prefecture, and Pingbian and Hekou counties) and other parts of Giangxi Autonomous Region (Jinxiu County) of China are anticipated.

Natural history notes. Prior to this study, the biological data of *Rhacophorus napoensis* were very limited; for example, it was only reported from an altitude of 1.032 m a.s.l. (Li et al. 2022). The species appears to be closely associated with secondary and evergreen forests with a wide range of altitudes of 400–430 mm a.s.l. from Bac Giang Province, at 870 m a.s.l. from Thanh Hoa Province, and 654–900 m a.s.l. from Nghe An Province (Hecht et al. 2013; Pham et al. 2016; this study). In Tay Yen Tu NR, Bac Giang Province, the frogs were found on trees and bushes, ca. 0.5–1.5 m above ground, near ponded water, in secondary forest. Other species of rhacophorids were recorded syntopically and included: Kurixalus cf. hainanus (Zhao, Wang & Shi), Polypedates megacephalus Hallowell, Rohanixalus cf. hansenae (Cochran), Theloderma albopunctatum (Liu & Hu), T. corticale (Boulenger), T. cf. lateriticum Bain, Nguyen & Doan, Zhangixalus pachyproctus Yu, Hui, Hou, Wu, Rao & Yang (Hecth et al. 2013; this study). In Xuan Lien NP., the frogs were found on trees and bushes, ca. 0.5–1.5 m above the ground, near ponded water, between 19:00 and 23:00. The surrounding habitat consisted of secondary forest composed of small to medium and sized hardwoods. Other species of rhacophorid were recorded syntopically and included: Gracixalus quangi Rowley, Dau, Nguyen, Cao & Nguyen, Kurixalus cf. hainanus, Raorchestes cf. malipoensis Huang, Liu, Du, Bernstein, Liu, Yang, Yu & Wu, Polypedates megacephalus, Rhacophorus kio Ohler & Delorme, R. orlovi Ziegler & Kohler, Theloderma albopunctatum, Zhangixalus pachyproctus (Pham et al. 2016; our data). In Pu Hoat NR, Nghe An Province, the frogs were found in a tree, ca. 0.5–2.0 m above the ground, more than 10 meters from the nearest stream, adjacent to a puddle in the secondary forest. The call of the frogs recorded in August is comprised of two calls types, 1–2 notes; calls types note 1, with 3–29 pulses, and calls types note 2, with 4–26 pulses of a call. The dominant frequency varied from 1,9–2,3 kHz. Other species of rhacophorids recorded syntopically included: Gracixalus quangi, Gracixalus sp., Raorchestes cf. malipoensis, Rhacophorus kio, R. orlovi, Theloderma albopunctatum, T. gordoni Taylor, Zhangixalus feae (Boulenger), Z. pachyproctus (Dau et al. 2012; our data). In Pu Mat NP, Nghe An Province, the frogs were found calling from leaves or branches 1–2.5 m above ground, near a ponded water, between 19:00 and 21:00. The surrounding habitat consisted of secondary forest composed of medium to larger hardwoods. During the breeding season, which lasted from May-August, this species breeds in rain pools and standing water in streams within forests. Foam nests were created on tree branches that overhang shallow, moving water (TV Nguyen pers. obs.). Other species of rhacophorids recorded syntopically included: Kurixalus cf. hainanus, Kurixalus gracilloides Nguyen, Duong, Luu & Poyarkov, *Polypedates megacephalus* Hallowell, Rohanixalus cf. hansenae, Raorchestes cf. malipoensis, Rhacophorus kio, R. orlovi, Theloderma albopunctatum, T. lateriticum, T. gordoni Taylor, and Zhangixalus dennysi (Blanford) (Nguyen et al. 2020a; this study)

Discussion

Rhacophorus napoensis was known previously only from Guangxi Autonomous Region, China. Based on our data and the available literature, this study provides the first records, of *R. napoensis* outside of China, from Vietnam (Bac Giang, Thanh Hoa, Nghe An, and Ha Tinh provinces). These new findings highlight that our knowledge of the herpetofauna of Vietnam, particularly of the border region between China and Vietnam (see Poyarkov et al. 2021, 2023; Nguyen et al. 2024), is still incomplete and that additional field research is required.

Presently, the range of *Rhacophorus bipunctatus* s. str. should be restricted to Northeastern India, Bangladesh, Bhutan, and Northern and Western Myanmar, while the range of R. rhodopus s. str. should be restricted to Southern China, Central and Southern Myanmar, Thailand, Northern and Northwestern Laos, Northwestern Vietnam, Cambodia, and Western (Peninsular) Malaysia (Bordoloi et al. 2007; Chan et al. 2018; Chen et al. 2022; this study). Consequently, herein we speculate that the records of these two species from the southeastern part of Yunnan Province and areas except Napo County in Guangxi Autonomous Region of China as reported by Yu et al. (2008a), Fei et al. (2009, 2012), AmphibiaChina (2024); Northern and North-Central Vietnam, including Ha Giang, Ha Noi, Lai Chau, Lao Cai, Son La, Phu Tho, Quang Binh, and Quang Tri provinces, which as reported by Nguyen et al. (2008), Nguyen et al. (2009), Hecht et al. (2013), Nguyen et al. (2014), Pham et al. (2016), Pham et al. (2017a,b), Tran & Le (2019, 2021), and Luong et al. (2021), and from central Laos in Nakai-Nam Theun NP, Khammuoan Province as reported based on specimen NCSM 85400 (http://portal.vertnet.org/o/ncsm/ncsmherp?id=2d83b252-923a-4689-866b-2253f1821e00); in Viengthong, Bolikhamxay Province as reported based on specimen NCSM 80909 (http://portal.vertnet. org/o/ncsm/ncsm-herp?id=22b8590b-786d-4bab-8cbca2761263dc4e); and Phou Samsoum Mt. in Xiangkhoang Province as reported by Nguyen et al. (2020a) should be assigned to *R. napoensis*. Re-examinations of specimens of *R. bipunctatus* and/or *R. rhodopus* from Southeastern region of Yunnan Province and areas except Napo County of Guangxi in China, Northern and North-Central Vietnam as well as from Central Laos are required to verify these distributions.

On other hand, although reported to be widely distributed in Vietnam, the actual distribution of R. rhodopus in Vietnam is still quite vague. According to literature, we only confirm the record *Rhacophorus rhodopus* in Northwest Vietnam based on morphological evidence by Nguyen et al. (2015b). Nguyen et al. (2015b) reported on the *R*. rhodopus from Muong Nhe NR, Dien Bien Province with SVL 33.3–36.4 mm (n=5) in males, 45.7–49.0 mm (n=3) in females, TbL/SVL 0.52–0.53 in males, 0.51–0.53 in females. These ratios are consistent with the identification characteristics of the species *R. rhodopus* (see Suppl. material 1: table S2) and this location Muong Nhe NR is very close to the type locality of R. rhodopus (123 km from northwest of Mengyang Town, Yunnan Province, China). Therefore, we recommend that the population at Muong Nhe NR, Dien Bien Province, Vietnam is still assigned to the species R. rhodopus, although future molecular studies are required to verify this.

Futhermore, the Rhacophorus namdaphaensis Sarkar & Sanyal, 1985 (type locality: Miao, Tirap District, Arunachal Pradesh Sate, India) was subsequently considered as a junior synonym of R. rhodopus according to Bordoloi et al. (2007). However, the individuals were found in Arunachal Pradesh State, in northeastern India, far from the type locality of *R. rhodopus* in southwestern China, and according to our observations, the morphology of the individuals of this species from Arunachal Pradesh seems to be more closely related to R. bipunctatus than to R. rhodopus (see Suppl. material 1: fig. S1D) with the presence of yellow pigmentation at the upper and lower portions of the iris, which are considered diagnostic characteristics of R. bipunctatus (see Suppl. material 1: figs. S1, S2). In addition, the area of distribution of *R. namda*phaensis is within the range of R. bipunctatus (Arunachal Pradesh Sate, India). Therefore, additional sampling of *R*. namdaphaensis at the type locality, with subsequent molecular and morphological analyses should be conducted to elucidate its taxonomic status. Futhermore, our molecular results confirm that previous findings of populations of R. cf. rhodopus from the Kon Tum-Gia Lai Plateu, Vietnam together with those from Hainan, China, as well as R. cf. rhodopus from Xizang, China, likely represent cryptic species in this complex, as revealed by other recent studies (Nguyen et al. 2014; Chan et al. 2018; Chen et al. 2022; Li et al. 2022). Therefore, future molecular and morphological studies and phylogenetic studies are required to clarify the taxonomy of the R. bipunctatus and R. rhodopus species complexes.

According to the original description of *Rhacophorus* napoensis by Li et al. (2022), this species is characterized by head width greater than head length (HW>HL), tibia length is slightly greater than a half of snout-vent length

(TbL/SVL 0.50–0.51 [avg. 0.50, n=5] in males), and by the absence of nuptial pad in males. However, the specimens from Vietnam examined in this study have head width almost equal to head length (HW≥HL), tibia length less than a half of snout-vent length (TbL/SVL 0.45–0.49 [avg. 0.47, n=9] in males, 0.43–0.46 [avg. 0.44, n=2] in females); nuptial pad is present on finger I in males. Our results suggest that these characters appear to be quite variable, so they cannot be used as diagnostic characters of *R. napoensis*.

Rhacophorus napoensis is to date reliably known from seven localities including two national parks, four nature reserves in Northern and North-Central Vietnam, as well as one narrow region in Southern Guangxi Autonomous Region, China. It is an arboreal species recorded from closed-canopy evergreen forests and forest edges in lowland and montane regions of rainforest. The main threats to this species in Vietnam are habitat loss and degradation, and harvesting for food (TV Nguyen pers. obs.). Though the actual extent of distribution, population trends, reproductive behaviour and ecology of this species remain poorly known, given the available information, we suggest *Rhacophorus napoensis* be considered of Least Concern (LC) following IUCN's Red List categories (IUCN 2019).

The new record of *Rhacophorus napoensis* in this study raises the known number of the genus *Rhacophorus* species in Vietnam to 17 with nine species being endemic in this country namely: R. helenae, R. hoabinhensis, R. hoanglienensis. R. larissae, R. marmoridorsum, R. trangdinhensis, R. vanbanicus, R. vampyrus, and R. viridimaculatus (Poyarkov et al. 2021; Frost et al. 2024; this study). Despite recent progress, the taxonomy and distribution of the species of *Rhacophorus* species in Vietnam still remain unclear, especially of the species belonging to R. hoanglienensis-orlovi species complex (including: R. hoanglienensis. R. larissae, R. marmoridorsum, R. trangdinhensis, R. vanbanicus, and R. viridimaculatus); many of these species lack molecular data and their taxonomic status remains questionable. Further field surveys and taxonomic efforts both in Vietnam and in the adjacent parts of Southeast Asia and China will likely reveal additional lineages within the widely-distributed and insufficiently sampled species of the genus *Rhacophorus*.

Acknowledgements

The fieldwork in Vietnam was completed within the frameworks and with partial financial support from the research project E-1.2 of the Joint Vietnam-Russia Tropical Science and Technology Research Centre for 2017. Permission to conduct fieldwork in Bac Giang Province was granted by the Bureau of Forestry, Ministry of Agriculture and Rural Development of Vietnam and by local administration (Peoples' Committee of Bac Giang Province: #712/TCLN-BTTN of 17.05.2017). We would like to thank the management board of Tay Yen Tu NR in Bac Giang Province; Pu Hoat NR, Pu Huong NR, and Pu Mat

NP in Nghe An Province, Vietnam for their permission to work in the national reserves and the park. We are deeply grateful to Abhishek Jamalabad, Aditya Satish, Jayanta Kr Roy (India), Paul Freed (USA), Pawangkhanant Parinya (Thailand), Peter Brakels (Laos), Guangyu Liu, Ming Jue, Chenxi Liao, Cui Yu (China), Tim Adriaens (Belgium), Artur Tomaszek (Hongkong), Thomas Bader (Austria), and Bang Van Tran (Vietnam) for providing photos of Rhacophorus spp. We also warmly thank Ngoc Quynh Nguyen (SIFASV, Vietnam) for help in the preparation of the figures. We are grateful to Christopher Oldnall for English proofreading and linguistic help. We thank Ben Wielstra, Gabriel Hershman and an anonymous reviewer for kindly reviewing a previous version of the manuscript. NAP and AVT are grateful to Andrei N. Kuznetsov, Svetlana P. Kuznetsova (JVRT STRC), Hoi Dang Nguyen (JVRT STRC), and Leonid P. Korzoun (MSU) for support and organization of fieldwork. We thank Valentina F. Orlova and Roman A. Nazarov (ZMMU) for permission to examine specimens under their care. This research was supported in part by the Rufford Foundation (Grant No. 39897-1) and the Russian Science Foundation (RSF grant No. 22-14-00037, molecular and phylogenetic analyses).

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Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: **table S1.** Measurements (in mm) of the snoutvent length (SVL) and tibia length (TbL) of the *Rhacophorus* bipunctatus, R. rhodopus, and R. napoensis from this study and the literature. table S2. Morphological comparisons of Rhacophorus napoensis with R. bipunctatus and R. rhodopus. Remark: N/a= data not available, *= request verification). table S3. List of localities of the Rhacophorus napoensis appearing on Fig. 1. Remark: ?= verification required. fig. S1. Rhacophorus bipunctatus complex in life (all not collected except CAS 229893): R. bipunctatus - A: Kongthong, Meghalaya, India; B: Nongpoh, Meghalaya, India; C: Shillong, Meghalaya, India; D: Roing, Arunachal Pradesh, India; E: Manas NP, Assam, India; F: Lawachara NP, Sylhet, Bangladesh; G: Putao, Kachin, Myanmar (CAS 229893, Holotype of R. htunwini); H: Myitkyina, Kachin, Myanmar; R. cf. bipunctatus – I: Linzhi, Xizang, China. Photographs by: A. Jamalabad (A), A. Satish (B), A. Sanglyne (C), J.K. Roy (D), S. Harikrishnan (E), P. Freed (F), H. Win (G), P. Pawangkhanant (H), and C. Yu (I). **fig. S2.** Rhacophorus rhodopus complex in life (all not collected): R. rhodopus – A: Mengla, Yunnan, China; B: Xishuangbanna, Yunnan, China; C: Doi Inthanon NP., Chiangmai, Thailand; D: Suan Phueng, Ratchaburi, Thailand; E: Phou Samsoum Mt., Xiangkhouang, Laos; F: Cameron Highlands, Pahang, Malaysia; G: Fraser's Hill, Pahang, Malaysia; R. cf. rhodopus – H: Jianfengling NP, Hainan, China, I: Tay Giang, Quang Nam, Vietnam. Photographs by: G.Y. Liu (A), J. Ming (B), T. Adriaens (C), P. Pawangkhanant (D), P. Brakels (E), T. Bader (F), A. Tomaszek (G), C.X. Liao (H), and B.V. Tran (I).

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Link: https://doi.org/10.3897/herpetozoa.37.e122317.suppl1